RESISTANCE OF RHABDITIS TO ACIDS.

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As is well known, *Anguillula aceti* lives in vineger and is highly resistant to acetic acid. Henneberg (1) has shown that this nematode can live in 13.5 per cent. acetic acid. Abbott and Richards have reported that it can live for more than 24 hours in Tellyesnicky's fluid, and for three or four hours in Gilson's fluid.

The writer intended to see in the first place whether other free-living nematodes, which do not come in contact with acids in their natural life, were resistant particularly to acetic acid as well as other acids. For this purpose *Rhabditis elegans* was chosen. In the course of the work it was found that *Rhabditis* is more resistant than the tadpole, *Daphnia*, *Aelosoma*, and *Paramecium* not only to various kinds of acids, but to other toxic substances as well. The cuticle with which nematodes are covered is known to be composed of a very resistant substance; experiments, therefore, were tried to determine whether or not the cuticle is responsible for this resistance, the worms, injured and uninjured, being stained in solutions of neutral red and of methylene blue. This work was begun at the suggestion of Dr. M. H. Jacobs, whom the writer wishes to thank for valuable advice.

Adult hermaphrodites of *Rhabditis elegans* and tadpoles about 10 mm. long and with external gills were used. *R. elegans* is found in decayed matter and is easily cultivated in a peptone solution. Every experiment was repeated at least twice, and 14 to 30 individuals were used for each experiment. The temperature at which experiments were done varied from 21° C. to 26° C.

As shown in Table I., *Rhabditis* is the most resistant to various kinds of acids. It might be noted that it can live about two hours in N/30 acetic acid, while *Daphnia*, the form next in order of resistance, can live only about an hour and a quarter in N/100 acetic acid. From the results of the experiments given in the table we notice, however, that *Rhabditis* is not particularly resistant to acetic acid as compared with other acids.

TABLE I.

AVERAGE TIME IN MINUTES REQUIRED TO CAUSE CESSATION OF ALL MOVEMENTS.

	Rhabditis.	*Paramecium.*	*Aelosoma.*	*Daphnia.*	*Tadpole.*
HCl, N/100	60	<1	<1	23	12
HCl, N/50	17	<1	<1	9	7
Acetic acid, N/10	23	<1	<1	6	—
Butyric acid, N/10	13	<1	<1	—	<1
Salicylic acid, N/100	26	<1	<1	13	—

Is this resistance of *Rhabditis* due to a mechanical reason or to the ability of the animal to neutralize acids? The results of the experiments with toxic substances (Table II.) seem to show that the first is the case, since *Rhabditis* is the most resistant of the five forms studied not only to acids, but to the other toxic substances as well. If this presumption is correct, the cuticle with which *Rhabditis* is covered may be supposed to prevent the penetration of the toxic substances.

TABLE II.

AVERAGE TIME IN MINUTES REQUIRED TO CAUSE CESSATION OF ALL MOVEMENTS.

	Rhabditis.	*Paramecium.*	*Aelosoma.*	*Daphnia.*	*Tadpole.*
NaOH N/40	38	<1	<1	15	2
Ether 5 per cent.	After 10 all revived	<1	<1	After 10 none revived	After 10 none revived
$HgCl_2$ 0.05 per cent.	60	<1	<1	25	5

We notice in these tables that *Rhabditis* is about 2.5 times as resistant to N/40 NaOH, 0.05 per cent. mercuric chloride and N/100 HCl than is *Daphnia*, the next most resistant form. Tadpoles died more quickly in N/20 NaOH than in N/20 HCl. As shown in Table II., tadpoles died in 2 minutes in N/40 NaOH, while they died in about 4 minutes in N/30 HCl. This is probably due to the fact that the acid coagulates the body proteins as it penetrates, while the NaOH dissolves them. Tadpoles, which were placed in the 5 per cent. solution of ether for 5 minutes, revived though they were in a very bad condition. None of the *Rhabdites* revived after placing them 30 minutes in the solution of ether.

In order to study the penetration of an intra-vitam stain, some

Rhabdites were placed in a small quantity of neutral red solution on a slide, and were observed under a microscope. The stain entered through the mouth, and soaked out through the wall of the esophagus. In one worm the stain entered through the anus, but only a small region of the posterior part of the body was stained.

A solution of neutral red, which stained dead worms deeply, stained living worms only faintly; a strong solution, therefore, was used. Staining for 2.5 hours in such a strong solution, the anterior part of the body as faras the second bulb was deeply stained, but in the remaining part the intestine only was colored. The intestine was stained less deeply than the anterior regions of the body, and the stain became progressively lighter toward the posterior end. This seems to show that the stain entered through the mouth, but not through the cuticle nor through the anus. After staining for 20 hours the worms were still alive, and the anterior regions a little beyond the bulb of the esophagus were deeply stained. In the remaining part the intestine only was colored, and its posterior regions were stained less deeply than its anterior regions.

Such stained worms were placed in a weak solution of NaOH, and then the alkali entered through the mouth, and proceeded towards the interior. In about 20 minutes the portion of the body as far as the second bulb became yellow, but the change in color did not go further. Later a rapid diffusion of NaOH from the posterior end anteriorly was observed, and thus the entire body of the worm changed to yellow; the alkali seemed to enter through the anus. In another worm the change in colour proceeded anteriorly starting from the anus.

To show definitely that the cuticle prevents the entrance of the stain and the NaOH, the posterior regions of some worms were cut off. Then the stain entered through the mouth and the cut end. Such worms were, therefore, stained in toto for 4 hours, and at the end of this time they were still alive. Contrary to the case of uninjured worms the change in color carried by NaOH began at both ends, and proceeded faster anteriorly from the cut end than from the mouth. In about one hour and three quarters the change in color has been completed in such injured worms.

As in the case of neutral red, methylene blue entered through the mouth, and in no case penetrated through the cuticle. To stain the entire body of Rhabditis a strong solution was used for the same reason which has been stated in the case of neutral red. After staining 24 hours, some worms were still alive, but others were dead. The anterior part of the living worms as far as the second bulb was deeply stained, but the remaining part was lighter in colour.

Such stained worms were placed in 83 per cent. alcohol. The disappearance of the color began at the anterior end. The worms soon died in the alcohol, and the latter then entered through the anus. As far as the second bulb of the esophagus or a little beyond it, the disappearance of the color was due to the alcohol which entered through the mouth; in the remaining regions to that which entered through the anus. A small region of the anterior part of the intestine still remained stained after one hour in alcohol. In one worm I observed that the posterior division of the hermaphrodite reproductive organs was still stained, while the posterior region of the intestine which is found beside them was already reduced in color.

As in the case of neutral red, in some worms whose posterior ends had been cut off, the stain and alcohol entered through the mouth and the cut end.

Summary.

1. *Anguillula aceti* is highly resistant to acetic acid. To see what is the case in other free-living nematodes *Rhabditis elegans* was used, and it was found that *Rhabditis* is much more resistant not only to various kinds of acids, but to other toxic substances also, as compared with the tadpole, *Daphnia*, *Aelosoma* and *Paramecium*.

2. When animals were stained in neutral red and in methylene blue, no case in which the stain entered through the cuticle was observed.

3. The entrance of NaOH and alcohol through the cuticle also could not be observed.

4. The stains, NaOH and alcohol entered through the mouth, and when the anus and vulva were opened, entered through them as well.

5. In the case of worms whose posterior ends had been cut off these substances in question entered through the cut end, as well as the mouth.

6. The conclusion to be drawn from these results seems to be that the impermeability of the cuticle is responsible for the resistance of *Rhabditis*.

LITERATURE CITED

1. Henneberg, W.
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